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HARMONIZATION OF EUROPEAN PHARMACOPEIAL AND US PHARMACOPEIAL METHODS OF GLIMEPIRIDE BY HPLC

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ABSTRACT

Glimepiride is official in both EP and USP. For analysis of this product three High performance liquid chromatography methods are reported in EP as well as USP. These methods are Assay, Related substances analysis and analysis of cis impurity (by chiral HPLC). This research paper includes the harmonization of three methods with respect to EP and USP monographs. This will lead to common analysis method for both EP as well as USP, saving time and cost of analysis. The harmonized methods are validated as per International Conference on Harmonization guidelines.

KEYWORDS: Glimepiride, Related substances, Assay, Chiral and High performance liquid chromatography, Harmonization and Cost saving.

INTRODUCTION

Glimepiride is a sulphonylurea agent that stimulates insulin release from pancreatic β-cells and may act via extrapancreatic mechanisms. It is administered once daily to patients with type 2 (non-insulin-dependent) diabetes.

The product is official in EP and USP, Indoco as a manufacturer of Glimepiride has both CEP and US DMF for this product. Hence the product has to be analyzing separately for EP as well as USP monographs. Even though many parameters of EP and USP methods are similar, however due to significance difference in system suitability parameter and other chromatographic conditions the separate analysis as per EP and USP monograph is required.

Harmonization of these methods with respect to EP and USP will save 50% time, cost of analysis and instrument occupancy.

Objectives of the study

The objective of the study was to harmonize EP and USP methods for the analysis of Glimepiride related substance and assay to save the analysis time and cost and also to demonstrate that the procedure when correctly applied produces results that are fit for the purpose.

Procedure/ Process

Materials and Reagent

Impurity standards were procured from USP. Whereas Sodium dihydrogen phosphate, Acetonitrile, Ortho-phosphoric acid, Heptane, Isopropyl alcohol, acetic acid and Methylene dichloride were procured from Merck Ltd., Sample of Glimepiride was obtained from Indoco Remedies Ltd., Navi Mumbai, India.

Apparatus

10 mL, 20 mL, 50 mL and 100 mL with stoppers, Class A Volumetric Flasks, 1.0 mL, 5.0 mL Graduated pipettes**,** 1 L Graduated Cylinder for mobile phase preparation, 1L Bottle for mobile phase preparation and storage, Filter Assembly for mobile phase filtration.

Instrument and chromatographic conditions

1) For Harmonized Related Substances Method (Impurity B, C and D) and Assay

High performance liquid chromatography analysis was carried out on Waters system (Empower) equipped with 2998 PDA detector. A C18 column (Supersphere RP-18e 250 mm x 4 mm, 4µm) was used for chromatographic separations. The mobile phase consisting mixture of 0.5 g of Sodium dihydrogen phosphate diluted to 500 mL water (pH 2.5 adjusted with Ortho phosphoric acid) and Acetonitrile (500:500).

2) For Harmonized Related Substances Method (Impurity A)

High performance liquid chromatography analysis was carried out on Waters system (Empower) equipped with 2998 PDA detector. A chiral column (Inertsphere diol-4 150 mm x 3 mm, 5µm) was used for chromatographic separations. The mobile phase consisting mixture of Isopropyl alcohol, Anhydrous acetic and Heptane (100:1:899).

Harmonization Of Methods

A) Preparation of system suitability solution and dilute standard solution for Related compound B,C and D test of Glimepiride

B) Mobile phase preparation for Glimepiride Related compound A.

Justification

A) Related compound B, C and D test of Glimepiride

For Reference Solution (a) / System suitability solution

In case of evaluation of system suitability for Assay and Related substances (Related Compounds) as per monograph of Glimepirirde EP 6.0 and USP 31, the preparation of solution for Reference solution a (System suitability solution in USP) are different.

As per USP 31, System suitability solution was prepared by weighing accurately 1 mg each of Glimepiride impurity B USPRS, Glimepiride impurity C USPRS, Glimepiride impurity D USPRS, in to 10 ml volumetric flask, dissolved in 5.0 ml of solvent mixture & had made up to volume with solvent mixture. Transferred 1.0 ml of this solution to 50.0 ml volumetric flask and made up to volume with the standard preparation. The resolution between peaks due to the impurity B and Impurity C should not be less than 4.0 obtained with above solution.

As per EP 6.0 Reference solution was prepared by dissolving content of a vial of Glimepiride System suitability CRS (containing impurities B, C and D) in 2.0 ml of test solution and the resolution between peaks due to the impurity B and Impurity C should not be less than 4.0 obtained with above solution.

However an equivalency study of these solutions as per both USP and EP monograph was carried out and it was demonstrated that result of both solutions are same for the purpose of given system suitability parameters. Hence the system suitability solution preparation was harmonized in house and has been used throught validation and routine analysis which are as follows.

Reference solution (a)

Procedure: Transfer accurately 1 mg each of Glimepiride impurity B USPRS, Glimepiride impurity C USPRS, Glimepiride impurity D USPRS, in to a 10 ml volumetric flask and dissolve in 5.0 ml of solvent mixture & make up to volume with solvent mixture. Transfer 1.0

ml of this solution to 50.0 ml volumetric flask and make up to volume with the standard preparation.

For Reference Solution (b) / Diluted standard solution

In case of evaluation of impurities in Related substances (Related Compounds) as per monograph of Glimepirirde EP 6.0 and USP 27, the preparation of solution for Reference solution b (diluted solution 1in USP) are different.

As per USP 31, diluted standard 1was prepared by transferring 5.0 ml of test solution (20.0 Glimepiride diluted in 100 ml volumetric flask) to 100 ml volumetric flask and made up to volume with solvent mixture. Transferred 5 ml of this solution to 50 ml volumetric flask and made up to volume with solvent mixture (0.5 %). The quantification was done by area obtained with above solution.

As per EP 6.0 Reference solution (b) was prepared by transferring 1.0 ml of test solution (20.0 mg Glimepiride diluted in 100 ml volumetric flask) to 100 ml volumetric flask and made up to volume with solvent mixture. Transferred 1 ml of this solution to 10 ml volumetric flask and made up to volume with solvent mixture (0.1%). The quantification was done by area obtained with above solution. However an equivalency study of theses solutions was carried out and it was demonstrated that result obtained from both the solution are same for the purpose of content of impurities. Also linearity of Glimepiride was established from 0.05 % to 0.75 %.Hence the diluted standard 1 preparation was harmonized in house and has been used throught validation and routine analysis which are as follows.

Reference solution (b)

Transfer 5.0 ml of test solution (20.0 mg Glimepiride diluted in 100 ml volumetric flask) to 100ml volumetric flask and make up to volume with solvent mixture and mix. Transfer 5 ml of this solution to 50 ml volumetric flask and make up to volume with solvent mixture (0.5 %).

Preparation of solution for Related Substances (Impurity B, C and D)

Solvent mixture: Prepare a mixture of water and acetonitrile (1:4).

Standard preparation

Transfer an accurately weighed 20.0 mg of Glimepiride working standard to 100 mL volumetric flask. Dissolve in 50 mL of solvent mixture and make upto volume with the same.

System suitability solution/Reference solution (a)

Weigh accurately 1.0 mg each of Glimepiride impurity B USP RS, Glimepiride impurity C USP RS and Glimepiride impurity D USP RS into a 10 mL volumetric flask. Dissolve in 5 mL solvent mixture and make up to volume with solvent mixture. Transfer 1.0 mL of this solution to 50.0 mL volumetric flask and make upto volume with the standard preparation.

Test solution

Transfer about 20.0 mg of Glimepiride, accurately weighed, to a 100 mL volumetric flask, dissolve in 50.0 mL of solvent mixture and make upto the volume with the same.

Diluted test solution 1/Reference solution (b)

Transfer 5.0 mL of the test solution to 100 mL volumetric flask, dissolve in 10.0 mL of solvent mixture, mix and make upto volume with same solvent mixture. Transfer 5.0 mL of this solution to 50 mL volumetric flask and make upto volume with solvent mixture.

Diluted test solution 2/Reference solution (c)

Transfer 1.0 mL of diluted test solution 1 to 10 mL volumetric flask and make upto volume with solvent mixture.

B) Related compound A of Glimepiride

Mobile phase preparation for Related compound A

The method for determination of Glimepiride Related compound A (Impurity-A) is modified with respect to mobile phase i.e. Heptane instead of Hexane (as per USP). This change is incorporated for to release of batches by single analysis for both modified method and USP method. The equivalency study for the modified method was carried by analysing the test sample as per both USP and modified method. Results are compared of both analysis.

Preparation of solution for Related Substances (Impurity A)

System suitability stock solution

Dissolve about 1.0 mg of Glimepiride Related Compound A RS in 1.0 mL of Methylene dichloride. Add 3.0 mL of mobile phase, and mix.

System suitability solution

Transfer about 10 mg of Glimepiride working standard to a 20 mL volumetric flask, and dissolve in 5.0 mL of Methylene dichloride. Dilute with mobile phase to volume, and mix,

Transfer 5.0 mL of this solution to a separate flask, add 50 µL of the system suitability stock solution, and mix

Test solution

Transfer about 10 mg of Glimepiride sample to a 20 mL volumetric flask, and dissolve in 5.0 mL of Methylene dichloride. Dilute with mobile phase to volume, and mix.

Assay Test

For Glimepiride assay both USP and EP method are harmonized with respect to system suitability solution as prepared in above related substance method (Impurity B, C and D) and assay is performed in single harmonized method.

System suitability solution/Reference solution (a)

Weigh accurately 1.0 mg each of Glimepiride impurity B USP RS, Glimepiride impurity C USP RS and Glimepiride impurity D USP RS into a 10 mL volumetric flask. Dissolve in 5 mL solvent mixture and make up to volume with solvent mixture. Transfer 1.0 mL of this solution to 50.0 mL volumetric flask and make upto volume with the standard preparation.

Standard solution

Weigh accurately 20 mg of Glimepiride Working Standard/reference standard into a 100 mL volumetric flask. Dissolve in 10 mL solvent mixture and make up to volume with solvent mixture.

Test solution

Transfer about 20.0 mg of Glimepiride sample, accurately weighed, into a 100 mL volumetric flask, dissolve in 10 mL solvent mixture and make up to volume with solvent mixture.

RESULT AND DISCUSSION

After harmonization of methods, validation is performed as per ICH guideline with below given parameter and result are tabulated.

Method Validation

The validation work was conducted according to the ICH (International Conference on Harmonization) guidelines. The validated method parameters include Specificity, Limit of detection, Limit of quantitation, Linearity, Accuracy and Precision.

Specificity

The specificity of the HPLC method was determined by injecting individual impurities and sample spiked with all impurities. Wherein no interference is observed for any of the impurities and main peak also peak purity of impurities and main peak was passing. This study confirmed the stability indicating power of the HPLC assay and RS method.

Limit of detection and Limit of quantitation

The limit of detection and limit of quantitation was determined for related substance method by injecting each impurity from 50% to 150% of target concentration by linear regression method where values for all impurities were found by to be below 0.06 % with respect to sample.

Linearity

Linearity of the method was checked by plotting calibration curves between the peak areas versus the concentration of each impurity over the range LOQ to 150% of limit level w.r.t test concentration. While in Assay linearity is performed from 80% to 120 % of target concentration of analyte. The method showed linear response for all the impurities in RS and for Glimepiride in Assay method.

Accuracy

The accuracy of the method was determined by recovery spiking each impurity in sample from LOQ to 150% of target concentration and performing recovery study. While in Assay method recovery is found out at 80% to 120% of Glimmered target concentration.

Precision

Precision of the method was evaluated in terms of Repeatability and Intermediate precision. The Repeatability is determined by calculating the relative standard deviation (% RSD) of six replicate determinations of test solution. For Intermediate precision, six replicate determinations of test solution was injected on different day by different system and relative standard deviation (% RSD) was calculated.

Table 1: Analytical Data For Assay.

Parameter		Result
Linearity Range (%w.r.t sample)		80 % to 120%
Regression coefficient		0.9993
Repeatability (% RSD)		0.16
% Recovery	80 %	105.68
	100 %	105.78
	120 %	106.65

Table 2: Analytical Data For Related compound.

Parameter		Impurity A	Impurity B	Impurity C	Impurity D
LOD (% w.r.t sample)		0.005	0.017	0.007	0.007
LOQ (% w.r.t sample)		0.016	0.052	0.021	0.022
Linearity Range (%w.r.t sample)		0.016 to 1.203	0.052 to 0.60	0.021 to 0.15	0.012 to 0.30
Regression coefficient		0.9999	0.9999	0.9921	0.9994
Repeatability (% RSD)		0.85	1.97	-	-
% Recovery	50 %	97.62	103.93	101.56	104.37
	100 %	94.81	100.94	98.72	103.83
	150 %	95.07	102.58	100.39	105.82

Benefit to the organization/Individual

During analysis of Glimepiride as per EP and USP methods separately we will required 4 HPLC instruments with 4 chemist and 3 days for reporting, but when we analyze the sample by harmonized method our requirements will be cut down upto 50% with respect to EP and USP methods requirements.

If we consider 1000 rupees cost per injection, then our calculation will be as follows.

Test	Number of Injection			Cost per Injection		
	EP Method	USP Method	Harmonized method	EP Method	USP Method	Harmonized method
Assay	11	11	11	11,000	11,000	11,000
Impurity B,C & D	10	10	10	10,000	10,000	10,000
Impurity A	7	5	7	7000	5000	7000
Total Cost				28,000	26,000	28,000
				54,000		28,000

When we analyze Glimepiride sample by EP and USP method, the total cost of the analysis will be 54000 and by Harmonized method analysis cost will be 28000. Hence by using Harmonized method we can save upto 26000 per batch of Glimepiride.

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